(IMM). While IMM fusion is not obligatory following fusion of the OMM, it most often follows in a matter of seconds. Little is known about the coordination of this close coupling. Using photactivatable fluorescent proteins and time-lapse confocal microscopy we have been able to visualize and quantify OMM-IMM fusion coupling in vivo under a variety of conditions to discover regulation by two inter-related factors. First, in cells deprived of oxidative substrate, reintroduction of glutamine significantly increased the efficiency of coupling as measured by the distribution of coupling times and OMM-only fusion events. This enhancement correlates with mitochondrial ATP levels measured by a matrix-targeted genetically encoded reporter, and supported by a strong decoupling effect observed following oligomycin treatment. Second, cells treated with carefully calibrated levels of the ionophores valinomycin or nigericin show increased and decreased coupling efficiency, respectively. We hypothesize this effect is due to matrix volume dilation and constriction that affects the physical interaction of the OMM and IMM.

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Mitochondrial Dynamics in Neonatal and Adult Cardiomyocytes

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Mitochondrial function is central to heart physiology and pathology. The function of mitochondria is dynamically regulated by their fusion and fission in many cell types. However, the study of mitochondrial dynamics in cardiomyocytes (CM) has been difficult. Using photoactivatable (PA) fluorescent proteins we characterized the mitochondrial dynamics in normal neonatal and adult rat CM. CM were transduced by mitochondrial matrix targeted DsRed and PA-GFP encoding adenoviruses and evaluated by confocal miscroscopy 36-48 h post infection. With this approach, we studied an early developmental stage (neonatal) and fully differentiated (adult) CM. We also studied the same cells upon prolonged exposure to ethanol that is known to cause mitochondrial dysfunction and cardiomyopathy.

We show that mitochondria form a highly connected network in neonatal CM, and mostly discrete structures with some connectivity mostly in longitudinal orientation in adult CM. Neonatal CM displayed considerable fusion activity (0.25 events/25 square micrometers/min). Eighty two % of the events resulted in complete merge of the organelles, whereas 18% appeared as fusion followed by separation. By contrast, the fusion events were scarce in the adult CM. Mitochondrial movements were also more frequent and elapsed longer distances in neonatal than in adult CM.

Neonatal CM exposed to 50 mM ethanol for 48h showed 40% decrease in the network continuity and 75% decrease in the fusion event rate. In adult CM isolated from ethanol-fed (6 months) rats, mitochondrial continuity decreased to 50% of the control.

Thus, mitochondria are highly dynamic in neonatal CM. Some stable intermitochondrial connections allow content exchange in the adult CM but less fusion activity is present, probably due to the spatial restriction presented by the myofilaments. Chronic ethanol exposure suppresses mitochondrial dynamics in CM, which effect might provide a possible mechanism for the impaired contractility.

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Purine Nucleotides Similarly Regulate Uncoupling Protein 3 and 1

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ATP generation is fueled by an electrical potential across the inner mitochondrial membrane (IMM), which can be decreased by an uncoupling protein (UCP) facilitated proton leak. Apart from UCP1, which supports non-shivering thermogenesis, the function of the other UCPs, such as UCP3, remains unknown. In contrast to UCP1, few results on UCP3 regulation from studies on isolated mitochondria or liposomes imply that ADP/GDP has a stronger inhibitory effect on UCP3 than ATP/GTP. In light of the fact that both, UCP1 and UCP3 were found in brown adipose tissue, we now test whether UCP3 and UCP1 are regulated differently. For this we compare the inhibition of UCP1 and UCP3 by PNs of varying phosphorylation and concentration, using a system of planar bilayers reconstituted with recombinant protein and FAs (1). In contrast to liposomes, this enables us to directly apply the membrane potential necessary for UCP3 function under physiological conditions. These results show that ATP and not ADP is the most potent UCP3 inhibitor and this is likewise similar to UCP1 and UCP2 (2, 3). UCP3 conductance is more strongly inhibited by the same PN concentrations than is UCP1 conductance, and adenosine nucleotides are more effective inhibitors than guanosines. Our results demonstrate that inhibition of UCP3 is similar to that of UCP1. We anticipate these findings as a starting point to examine whether different factors (in comparison to UCP1) are required to activate UCP3.

(1) Beck et al. (2006) Biochim Biophys Acta. 1757(5-6):474-9.
(2) Beck et al. (2007) FASEB J. 21(4):1137-44.
(3) Rupprech et al. (2010) Biophys J. 98(8):1503-11.

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Quantification of Mitochondrial UCP3 Expression in Mouse Tissues

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The mitochondrial uncoupling protein family includes at least five members (UCP1 - UCP5), which are implicated in the pathophysiology of different diseases such as obesity, diabetes type II, ischemia, cancer and neurodegenerative disorders. In contrast to the well-defined function of UCP1 in thermogenesis, the uncertain role and expression patterns of subfamily members are controversially discussed. Recently, we suggested that UCP2 and UCP4 expression is tightly connected to a certain type of cell metabolism (1,2). Surprisingly, highly homologous UCP1 and UCP3 with similar proton transport functions were reported to be present in BAT. To get a hint about the reason for this expression pattern, we aimed to quantify the amounts of UCP3 in mouse tissues under different conditions in this study and compare it to the UCP1 amounts in BAT. For this we designed a specific antibody against UCP3, which we have validated using UCP3 knockout mice tissue and recombinant mouse UCP3. We confirmed that UCP3 is expressed in brown adipose tissue, gastrocnemius muscle, scapular muscle and the heart. Using an established WB approach with recombinant UCPs, we were able to show for the first time, that the amount of the expressed UCP3 in BAT is much higher compared to the muscle samples, but still considerably lower than the amount of UCP1 in BAT determined previously (1). UCP3 abundance in muscles fluctuates strongly in different mice and between various muscle types of the same mouse already under physiological conditions. The results of this study support the hypothesis about different biological function of both, UCP1 and UCP3.

1. Smorodchenko,A et al. Mol Cell Neurosci. 2011 Aug;47(4):244-53.
2. Rupprecht,A. et al. PLoS One. 2012;7(8):e41406.

2993-Pos Board B685

Mitochondrial Uncoupling and Thermogenesis in Beige Fat

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Beige adipocytes are a morphologically distinct type of fat cell that develop within white fat depots upon exposure to cold or high fat diet. These cells have emerged as a major contributor to adaptive thermogenenesis and control of obesity, along with brown fat, a classic thermogenic tissue. Indeed, similarly to brown adipocytes, beige adipocytes have been shown to express mitochondrial uncoupling protein 1 (UCP1), which is known to convert ATP into heat by increasing the passive proton (H^+) leak of the inner mitochondrial membrane. However, the mechanisms of mitochondrial uncoupling and thermogenesis in beige fat as well as the relative contribution of UCP1 still remain unclear.

Here we developed a method for isolation of a pure population of beige adipocyte mitochondria from white fat depots and for the first time directly characterized the mechanism of mitochondrial uncoupling in beige fat using the mitochondrial patch-clamp. We demonstrate that the inner membrane of beige fat mitochondria has very large H^+ conductance comparable to that found in brown fat. This H^+ conductance was fatty-acid dependent, inhibited by purine nucleotides, and disappeared completely in UCP1(-/-) mice. We conclude that UCP1 is responsible for mitochondrial uncoupling in beige fat, and that the thermogenic capacity of beige fat is similar to that of brown fat. This observation also confirms that beige adipocytes significantly contribute to adaptive thermogenesis, which makes them an attractive therapeutic target to treat obesity.

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UCP2 Overexpression Worsens Mitochondrial Dysfunction and Accelerates Disease Progression in a Mouse Model of Amyotrophic Lateral Sclerosis

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Mitochondrial dysfunction leading to deficits in energy production, calcium capacity, and free radical generation has been implicated in the pathogenesis of familial amyotrophic lateral sclerosis (ALS) caused by mutations in Cu,Zn